

**INVITED REVIEW: ANNATTO USAGE AND BLEACHING IN DAIRY FOODS**

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Annatto is a yellow/orange colorant that is widely used in the food industry, particularly in the dairy industry. Annatto, consisting of the carotenoids bixin and norbixin, is most commonly added to produce orange cheese, such as Cheddar, to achieve a consistent color over seasonal changes. This colorant is not all retained in the cheese, and thus a percentage remains in the whey, which is highly undesirable. As a result, whey is often bleached. Hydrogen peroxide and benzoyl peroxide are the 2 bleaching agents currently approved for bleaching whey in the United States. Recent studies have highlighted the negative effect of bleaching on whey flavor while concurrently there is a dearth of current studies on bleaching conditions and efficacy. Recent international mandates have placed additional concern on the use of benzoyl peroxide as a bleaching agent. This review discusses the advantages, disadvantages, regulatory concerns, flavor implications, and optimal usage conditions of 2 widely used bleaching agents, hydrogen peroxide and benzoyl peroxide, as well as a few alternative methods including lipoygenase, peroxidase, and lactoperoxidase systems.

**A METHODOLOGY FOR MONITORING GLOBULAR MILK PROTEIN CHANGES INDUCED BY ULTRAFILTRATION: A DUAL STRUCTURAL AND FUNCTIONAL APPROACH**

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Understanding filtration mechanisms at a molecular level is important for predicting structural and functional properties of globular milk proteins after membrane operations. This stage is thus highly decisive for the further development of membrane separations as an efficient alternative to chromatographic processes for the fractionation of milk proteins. In this study, we proposed an original and complete analytical package for the examination of the putative effect of filtration at both macroscopic and molecular levels. We then investigated the pertinence of this analytical package during ultrafiltration (UF) of globular milk proteins in both dead-end and crossflow modes. Reverse-phase HPLC combined with statistical computing was shown to be relevant for the assessment of even slight physically induced modifications. Adaptations of circular dichroism and solubility measurements, regarding

their respective dependence on temperature and pH, were also useful for an accurate evaluation of functional modifications. At last, immunochemistry was proven to be a pertinent tool for the specific detection of modifications affecting a targeted protein, even in mixed solutions. Moreover, results obtained by such methods were shown to be coherent with data obtained from classical techniques such as fluorescence. For  $\beta$ -lactoglobulin, some physically induced modifications were noticed in the permeate because of shear stress inside membrane pores. In the case of  $\alpha$ -lactalbumin dead-end UF, permeation was shown to affect protein characteristics because of an increase in the relative calcium content responsible for a conformational transition from the apo-form to the holo-form of the protein. Finally, during crossflow UF with limited transmission of BSA, observations were coherent with a partial aggregation because of the circulation of proteins in the filtration pilot. Such a hypothesis corroborates results previously mentioned in the literature.

#### **SHORT COMMUNICATION: RAPID ANTIBIOTIC SCREENING TESTS DETECT ANTI-BIOTIC RESIDUES IN POWDERED MILK PRODUCTS**

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Rapid antibiotic screening tests are widely used in the dairy industry to monitor milk for the presence of antibiotic residues above regulated levels. Given the persistent concern over contamination of milk products with antibiotic residues, we investigated the utility of IDEXX Snap test devices (IDEXX Laboratories Inc., Westbrook, ME) as tools for detecting antibiotic residues in powdered milk products. Five powdered milk products were reconstituted according to manufacturer specification with distilled water: Carnation (Nestlé USA Inc., Solon, OH), Nido youth and Nido adult (Nestlé Mexico Inc., Mexico City, Mexico), ELK (Campina, Eindhoven, the Netherlands), and Regilait (Saint-Martin-Belle-Roche, France). Positive samples were generated by spiking reconstituted milk with penicillin G, cephapirin, or tetracycline to either the European Union-regulated maximum residue limit or the FDA-regulated safe/tolerance level, whichever was lower. Control, unspiked negative milk samples and positive samples were tested with appropriate IDEXX Snap test kits (penicillin G and cephapirin with New Beta-Lactam, tetracycline with New Tetracycline). All samples yielded definitive results consistent with expectations, and there were no instances of false-positive or false-negative readings. These results suggest that both the New Beta-Lactam and New Tetracycline IDEXX Snap test kits effectively detect antibiotic residues in commercially available powdered milk samples and are useful tools for monitoring antibiotic residues in reconstituted powdered milk products.

#### **COMPARISON OF COMPOSITION AND SENSORY PROPERTIES OF 80% WHEY PROTEIN AND MILK SERUM PROTEIN CONCENTRATES**

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Milk serum protein concentrates (SPC) are proteins found in cheese whey that are removed directly from milk. Because SPC are not exposed to the cheese-making process, enzymatic or chemical reactions that can lead to off-flavors are reduced. The objectives were to identify and compare the compo-

sition, flavor, and volatile components of 80% protein SPC and whey protein concentrates (WPC). Each pair of 80% SPC and WPC was manufactured from the same lot of milk and this was replicated 3 times. At each replication, spray-dried product from each protein source was collected. Commercial 80% WPC were also collected from several manufacturers for sensory and volatile analyses. A trained sensory panel documented the sensory profiles of the rehydrated powders. Volatile components were extracted by solid-phase microextraction and solvent extraction followed by solvent-assisted flavor evaporation with gas chromatography-mass spectrometry and gas chromatography-olfactometry. Consumer acceptance testing of acidified 6% protein beverages made with 80% SPC and WPC produced in the pilot plant and with WPC from commercial sources was conducted. The SPC was lower in fat and had a higher pH than the WPC produced in the pilot plant or commercial WPC. Few sensory differences were found between the rehydrated SPC and WPC manufactured in this study, but their flavor profiles were distinct from the flavor of rehydrated commercial WPC. The pilot-plant WPC had higher concentrations of lipid oxidation products compared with SPC, which may be related to the higher fat content of WPC. There was a large difference in appearance between 80% SPC and WPC: solutions of SPC were clear and those of WPC were opaque. Concentrations of lipid oxidation products in commercial WPC were generally higher than those in pilot-plant SPC or WPC. Sensory profiles of the peach-flavored protein beverage included cereal, free fatty acid, and soapy flavors and bitter taste in beverages made from pilot-plant products, whereas cardboard flavors were detected in those made with commercial WPC. Consumer liking scores for the beverages made with SPC were ranked highest or equally high with beverages made with WPC for aroma, appearance, and mouthfeel, but the beverages made with SPC had lower flavor and overall liking scores compared with beverages made with 3 of the 4 WPC.

#### **ROLES OF CHARGE INTERACTIONS ON ASTRINGENCY OF WHEY PROTEINS AT LOW PH**

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Whey proteins are a major ingredient in sports drink and functional beverages. At low pH, whey proteins are astringent, which may be undesirable in some applications. Understanding the astringency mechanism of whey proteins at low pH could lead to developing ways to minimize the astringency. This study compared the astringency of  $\beta$ -lactoglobulin ( $\beta$ -LG) at low pH with phosphate buffer controls having the same amount of phosphate and at similar pH. Results showed that  $\beta$ -LG samples were more astringent than phosphate buffers, indicating that astringency was not caused by acid alone and that proteins contribute to astringency. When comparing among various whey protein isolates (WPI) and lactoferrin at pH 3.5, 4.5, and 7.0, lactoferrin was astringent at pH 7.0 where no acid was added. In contrast, astringency of all WPI decreased at pH 7.0. This can be explained by lactoferrin remaining positively charged at pH 7.0 and able to interact with negatively charged saliva proteins, whereas the negatively charged WPI would not interact. Charge interactions were further supported by  $\beta$ -LG or lactoferrin and salivary proteins precipitating when mixed at conditions where  $\beta$ -LG, lactoferrin, or saliva themselves did not precipitate. It can be concluded that interactions between positively charged whey proteins and salivary proteins play a role in astringency of proteins at low pH.

**ROLE OF PROTEIN CONCENTRATION AND PROTEIN–SALIVA INTERACTIONS IN THE ASTRINGENCY OF WHEY PROTEINS AT LOW PH**

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Whey protein beverages are adjusted to pH <4.5 to enhance clarity and stability, but this pH level is also associated with increased astringency. The goal of this investigation was to determine the effects of protein concentration on astringency and interactions between whey and salivary proteins. Whey protein beverages containing 0.25 to 13% (wt/wt)  $\beta$ -lactoglobulin and 0.017% (wt/wt) sucralose at pH 2.6 to 4.2 were examined using descriptive sensory analysis. Controls were similar pH phosphate buffers at phosphate concentrations equivalent to the amount of phosphoric acid required to adjust the pH of the protein solution. Changes in astringency with protein concentration depended on pH. At pH 3.5, astringency significantly increased with protein concentration from 0.25 to 4% (wt/wt) and then remained constant from 4 to 13% (wt/wt). Conversely, at pH 2.6, astringency decreased with an increase in protein concentration [0.5–10% (wt/wt)]. This suggests a complex relationship that includes pH and buffering capacity of the beverages. Furthermore, saliva flow rates increased with increasing protein concentrations, showing that the physiological conditions in the mouth change with protein concentration. Maximum turbidity of whey protein–saliva mixtures was observed between pH 4.6 and 5.2. Both sensory evaluation and in vitro study of interactions between  $\beta$ -LG and saliva indicate that astringency of whey proteins is a complex process determined by the extent of aggregation occurring in the mouth, which depends on the whey protein beverage pH and buffering capacity in addition to saliva flow rate.

**THE IMPACT OF ANTIOXIDANT ADDITION ON FLAVOR OF CHEDDAR AND MOZZARELLA WHEY AND CHEDDAR WHEY PROTEIN CONCENTRATE**

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Lipid oxidation products are primary contributors to whey ingredient off-flavors. The objectives of this study were to evaluate the impact of antioxidant addition in prevention of flavor deterioration of fluid whey and spray-dried whey protein. Cheddar and Mozzarella cheeses were manufactured in triplicate. Fresh whey was collected, pasteurized, and defatted by centrifugal separation. Subsequently, 0.05% (w/w) ascorbic acid or 0.5% (w/w) whey protein hydrolysate (WPH) were added to the pasteurized whey. A control with no antioxidant addition was also evaluated. Wheys were stored at 3 °C and evaluated after 0, 2, 4, 6, and 8 d. In a subsequent experiment, selected treatments were then incorporated into liquid Cheddar whey and processed into whey protein concentrate (WPC). Whey and WPC flavors were documented by descriptive sensory analysis, and volatile components were evaluated by solid phase micro-extraction with gas chromatography mass spectrometry. Cardboard flavors increased in fluid wheys with storage. Liquid wheys with ascorbic acid or WPH had lower cardboard flavor across storage compared to control whey. Lipid oxidation products, hexanal, heptanal, octanal, and nonanal increased in liquid whey during storage, but liquid whey with added ascorbic acid or WPH had lower concentrations of these products compared to untreated controls. Mozzarella liquid whey had lower flavor intensities than Cheddar whey initially and after refrigerated storage.

WPC with added ascorbic acid or WPH had lower cardboard flavor and lower concentrations of pentanal, heptanal, and nonanal compared to control WPC. These results suggest that addition of an antioxidant to liquid whey prior to further processing may be beneficial to flavor of spray-dried whey protein.

### **ROLES OF WATER AND SOLIDS COMPOSITION IN THE CONTROL OF GLASS TRANSITION AND STICKINESS OF MILK POWDERS**

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Plasticization and glass transition of amorphous components in food powders often result in stickiness and caking. The glass transition temperature ( $T_g$ ) of milk powders was measured by differential scanning calorimetry (DSC) and a viscometer method was used to determine sticky-point temperatures. Water sorption isotherms were established for varying solids compositions. Lactose contents were analyzed by high-performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) and proteins were identified using SDS-PAGE gel electrophoresis. Solids composition and water affected both the  $T_g$  and stickiness behavior. Stickiness was governed by carbohydrates and water plasticization. At low protein contents, precrystallization of lactose decreased the sticky point temperature, but increasing protein content in all milk powders decreased stickiness at all water activities. The results showed that glass transition can be used to describe time-dependent stickiness and crystallization phenomena, and it can be used as a parameter to control and reduce stickiness of dairy solids with various compositions.

### **FORMATION OF ELASTIC WHEY PROTEIN GELS AT LOW PH BY ACID EQUILIBRATION**

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Whey protein gels have a weak/brittle texture when formed at  $\text{pH} = 4.5$ , yet this  $\text{pH}$  is required to produce a high-protein, shelf-stable product. We investigated if gels could be made under conditions that produced strong/elastic textural properties then adjusted to  $\text{pH} = 4.5$  and maintain textural properties. Gels were initially formed at 15% w/w protein ( $\text{pH} 7.5$ ). Equilibration in acid solutions caused gel swelling and lowered  $\text{pH}$  because of the diffusion of water and  $\text{H}^+$  into the gels. The type and concentration of acid, and presence of other ions, in the equilibrating solutions influenced  $\text{pH}$ , swelling ratio, and fracture properties of the gels. Swelling of gels decreased fracture stress (because of decreased protein network density) but caused little change to fracture strain, thus maintaining a desirable strong/elastic fracture pattern. We have shown that whey protein isolate gels can be made at  $\text{pH} = 4.5$  with a strong/elastic fracture pattern and the magnitude of this pattern can be altered by varying the acid type, acid concentration,  $\text{pH}$  of equilibrating solution, and equilibrating time.